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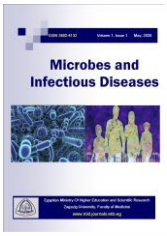

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Original article

Antimicrobial susceptibility pattern and molecular characterization of ESßLs producing *Salmonella enterica* isolated from patients attending hospitals in Northeast Nigeria

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ABSTRACT

Background: *Salmonella enterica* is a major cause of enteric fever worldwide resulting in thousands of deaths each year. This study aims to identify and characterize ESßLs-producing *Salmonella enterica* strains isolated from patients attending hospitals in Northeast Nigeria. **Material and methods:** In this cross-sectional analytical study, about 669 specimens of stool and blood were collected from patients aged between zero to 70 years, presenting with fever and gastrointestinal symptoms at three selected health facilities in Northeast Nigeria. The research was conducted from September 2020 to February 2021. A self-administered questionnaire was used to obtain demographic information from the subjects. Samples were cultured under standard microbiological protocols. Isolates obtained were identified using biochemical as well as polymerase chain reaction (PCR) targeting the 16S rRNA gene. A double disk diffusion synergy test was used to phenotypically evaluate the presence of ESßLs production. ESßLs genes were detected using multiplex PCR, amplicon size obtained was compared with 1kb ladder. **Results:** A total of nineteen (19) *Salmonella enterica* were discovered in the study, indicating a prevalence rate of 2.8%. These strains were resistant to at least three antibiotics, including Amikacin, Ampicillin, and Tetracycline. Phenotypic testing revealed ESßL activity in all 19 *Salmonella enterica* strains, but only 2 (10%) contained ESßL genes, as discovered in molecular analysis. The ESßLs genes determined were CMY-2, CTXM-1, and SHV. **Conclusion:** This study revealed burden of antimicrobial resistance and ESßLs genes in *Salmonella enterica* despite its low prevalence. Hence, screening for ESßLs in pathogenic bacteria is necessary in healthcare facilities.

Introduction

Salmonella enterica is a leading cause of gastroenteritis and bacteremia in humans globally [1]. In Africa, areas with high levels of epidemic and endemic typhoid and paratyphoid fever are increasingly being reported and identified as the leading cause of community-acquired bloodstream infection [2]. Nigeria is one of the tropical and subtropical nations where *Salmonella enterica* is

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most prevalent, and it is a major cause of morbidity and mortality there [3]. A wide range of animals, especially food animals, have been identified as non-typhoidal *Salmonella* reservoirs. Although approximately 2,500 *Salmonella enterica* serovars have been discovered, most human infections are caused by a small number of serovars [4]. The most common causes of human salmonellosis worldwide are *Salmonella enterica* serovars *Typhimurium* and *enteriditis.* However, other serovars are more prevalent in various places [5].

Antimicrobial resistance is a major global issue with many microbial organisms becoming resistant to drugs over the past 30 years [6]. There is growing concern about the emergence of multidrug-resistant phenotypes of *Salmonella* serotypes, such *as S. typhimurium, S. enteritidis,* and *S. Newport* [6]. The emergence of resistance to quinolones, fluoroquinolones, and extended spectrum cephalosporins like ceftiofur and ceftriaxone is particularly worrying [7]. The increasing resistance to cephalosporins, due to the production of extended-spectrum beta-lactamases (ESßLs), is a serious concern worldwide [8]. This has led to a shift in the choice of antibiotics worldwide [8]. In Nigeria, the emergence of resistance to third generation cephalosporins due to the acquisition and expression of ESßLs enzymes among *Salmonella* isolates has long been a common finding in hospital and community settings [9,10]. However, information on ESßLs producing *Salmonella enterica* serovars in Nigeria, particularly the Northeast region, remains inadequate. As such, this study aims to characterize ESßLs producing *Salmonella enterica* from patients attending selected tertiary hospitals in Northeast Nigeria.

Material and methods

Ethical consideration

The protocol for this study was approved by the ethical committees of the selected hospitals (Abubakar Tafawa Balewa University Teaching Hospital Bauchi, University of Maiduguri Teaching Hospital and Federal Teaching Hospital, Gombe).

Study area

The study was conducted in Northeast Nigeria, which is located between the arid Sahara and the lush tropical rainforest along the Guinea Coast. This region covers almost one-third (280,419 km^2) of Nigeria's land area (909,890 km^2) and is comprised of six states: Adamawa, Bauchi, Borno, Gombe, Taraba, and Yobe [11].

Study design

This study was conducted using a cross-sectional analytical approach. The plan was to identify patients between the ages of zero and seventy (70) who exhibited symptoms of pyrexia and gastroenteritis. Patients who did not show symptoms of these conditions were excluded from the study. All patients or their caregivers were given an informed consent form to sign, which sought their permission to participate in the study.

Sampling technique

A systematic random sampling technique was applied in the selection of the participants among eligible patients. The target population for this study included inpatients and outpatients attending general outpatient department of the selected hospitals.

Sample size determination

The size of the research sample size was determined using the following Cochran formula for estimating proportion: $n = Z^2pq/d^2$

Where: n= sample size of proportion

Z= standard score corresponding to a given confidence level at 95% confidence level (1.96)

P= prevalence from previous studies 0.169, q= 1-p, d= desired precision limit or proportion of sampling error 3%

$$n = \frac{1.96^2 \times 0.169 \times 0.85}{0.03^2} = 608.5,$$

608+10%= 608.5+60.8= 669

Data and sample collection

The subjects were asked to fill out a questionnaire to provide demographic information such as age, gender, hospital number, hospital name, ward/clinic, residential area, occupation, marital status, and presence of symptoms like gastroenteritis, pyrexia, or both pyrexia and gastroenteritis, as well as antibiotic exposure. A total of 669 blood and stool samples were collected from patients in selected hospitals between September 2020 and February 2021. For collecting stool specimens, individuals who were in their second or third week of illness were provided with clean containers and instructed to transfer approximately 2 grams of stool into tubes containing 8 milliliters of Selenite F Broth. These tubes were then incubated at 37°C for 24 hours. To collect

blood, a five-milliliter syringe was used and approximately 2 milliliters of blood were collected from patients who had developed fever during the first week of illness in a sterile manner [12].

Isolation and identification of bacteria

The stool samples from enrichment medium (Selenite–F broth) were sub-cultured unto *Salmonella-Shigella* agar (SSA), Chocolate agar (CA) and MacConkey agar (MCA) (Titan Biotech, India) and were incubated overnight at 37 ºC 18-24 hours and examined for growth [12]. The blood samples collected by peripheral veni-puncture were inoculated in Thioglycollate broth and then incubated overnight at 37 ºC for 18 to 24 hours. Tubes that showed turbidity were subcultured from each of the containers into freshly prepared *Salmonella-Shigella* agar (SSA) and MacConkey agar (MCA), Chocolate agar (CA) and incubated at 37 °C for 18-24 hours. Tubes that showed no turbidity were kept for 3 to 5 days before being sub-cultured [12]. All the isolates were subjected to Gram staining as per standard procedure identified using biochemical methods including urease test, indole test, hydrogen sulphide production test and sugar fermentation tests to detect the presence of the following sugars (mannitol, maltose, dulcitol, sucrose, and glucose). Isolates were further serotyped using O and H polyvalent sera (Bio-Rad, USA) [13].

Molecular identification of isolates using 16S rRNA sequencing

Amplification of the 16S rRNA gene was carried out using the generic 16S rRNA primers described by [14]. DNA was extracted from bacterial isolates using the boiling method with the Accu prep Genomic DNA extraction kit from (Bioneer, Korea). The PCR program was carried out in 50 µL reaction volumes containing 25 µL of EconoTaq® PLUS 2X Master Mix (Bioneer, Korea), 1 µL of DNA template (300 ng), 1 µL of 100 µM each primer (forward and reverse) and nuclease-free water marked up to the volume. The primers used in this study were RIBOSE-1 (GGACTACAGGGTATCTAAT 16S primer forward) and RIBOSE-2 (AGAGTTTGATCCTGG 16S Primer reverse) and were specific to a DNA fragment of 789 bp in size. Positive and negative DNA controls were performed in each reaction by adding 1 µL of approximately 100ng DNA template of *Salmonella enterica* and 10 µL of nuclease-free water, respectively, in the PCR assay. The 16S rRNA gene consisted of the initial DNA amplification carried out in a thermal cycler (plate_number_1), MJ Research serial no. 1433, using an initial denaturation step at 95°C for 5 min, followed by 30 cycles of amplification with denaturation at 94°C for 40 sec, annealing at 54°C for 40 sec, and extension at 72°C for 30 sec of 35 cycles, ending with a final extension at 72°C for 5 min. A negative control (NFW) was included in each PCR reaction to achieve PCR efficiency and to detect contamination [14].

Amplified PCR products were separated using 1.5% (w/v) agarose gel electrophoresis. Two microliters (2µL) of the loading dye were added to five microliters of the PCR products. An appropriate DNA size marker was loaded along with experimental samples. The gel electrophoresis was set at 100 V for 30 min and run until the dye had migrated to an appropriate distance. Subsequently, the gel was visualized under UV light through gel documentation of GeneSys GBOX (Syngene, USA). The size of the PCR products was checked using a 1 kb ladder **(**Thermo Fisher Scientific, USA**)** as standard [15]. The PCR product was later sent for sequencing.16S rRNA sequence data were theoretically confirmed through BLAST (Basic Local Alignment Search Tool) software analysis in the NCBI (National Center for Biotechnology Information) Genbank database available at http://www.ncbi.nlm.nih.gov/. The phylogenetic tree was constructed based on the sequence information using the Neighbor Joining tree on MEGA-X software [15].

Antimicrobial susceptibility test

The bacterial isolates were tested for antibiotic susceptibility using the Kirby-Bauer disc diffusion method [16]. In this method, lawn culture was made using culture suspension (adjusted to 0.5 McFarland's standards) of bacterial test isolates on the surface of Mueller–Hinton agar (MHA) plates (BD DIFCO^{TM}, USA). Standard antibiotics such as ampicillin (10 µg), amoxicillin/clavulanic acid (25/5 µg), amikacin (30 µg), ceftazidime (30µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamycin (10 µg), imipenem (10 µg), kanamycin (30 µg), meropenem (10 µg), sulphate colistin (10 µg), penicillin (30 µg), tetracycline (30 µg), cotrimoxazole (30 µg), sparfloxacin (5 µg), and levofloxacin (10 µg) (Oxoid, England) were used to test for the sensitivity of the isolates.

The diameters of the zones of inhibition were measured and interpreted by standards approved by the Clinical and Laboratory Standard Institute (CLSI) [17]. *E. coli* American Type Culture Collection (ATCC) 25922 was obtained from the National Veterinary Research Institute (NVRI), Vom, and was used as a control. Multidrug resistance phenotype was defined as resistance to three or more classes of antibiotics [9].

Detection of ESßLs genes

Phenotypic detection of ESßLs production

For detecting ESßL production, all the isolates that showed reduced susceptibility to third-generation cephalosporins (3GCs) were tested using the double disk synergy test method [18]. The method involved placing the 3GC antibiotics, namely ceftazidime (30 µg) and ceftriaxone (30 µg), at a distance of 15 mm (centre to centre) from amoxicillin/clavulanic acid (25/5 µg) using CLSI interpretative guidelines as the standard. A positive ESßL production was identified if there was a clear extension of the edge of the inhibition zone of any of the antibiotics towards the central disk (amoxicillin-clavulanic acid) or an increased zone of inhibition by ≥5 mm when used in combination. To ensure accuracy, *K. pneumoniae* 700603 was used as a control strain for a positive ESßL production, while *E. coli* 25922 was used as a negative control for ESßL production [9].

Molecular detection of ESßLs genes

The presence of genes encoding SHV, CTX-M1 and CMY-2 type β-lactamases were detected by multiplex PCR using specific primers and conditions previously reported [19,20].

A total reaction volume of 50 µL was prepared, which consisted of 2 µL of genomic DNA, 0.4 µM of each forward and reverse specific primers, 25 µL of 2X PCR Bestaq Master Mixes (Applied Biological Materials, Canada), and 19 µL of sterile de-ionized water. The PCR amplification was carried out on the BIORad thermal cycler (Bio-Rad, USA) with an initial denaturation step at 94 °C for 3 minutes, followed by 35 cycles of amplification with denaturation at 94 °C for 10 seconds, annealing at 61 °C for 30 seconds, and extension at 72 °C for 1 minute. The PCR process was completed by extending the final cycle at 72 °C for 5 minutes. The resulting PCR products were subjected to electrophoresis using a 1.5% agarose gel. Ethidium bromide (Sigma-Aldrich, USA) was used at a concentration of 1 µg/mL to stain the gel and the gel was then placed under a UV transluminator to visualize the amplicons. The DNA bands of each amplicon were photographed and compared against a 100-bp plus DNA mass marker [23].

Results

Isolation and identification of ESßLs *Salmonella enterica*

During the study, bacterial pathogens were identified and isolated based on various characteristics such as colonial morphology, Gram staining, and biochemical tests including catalase, coagulase, indole, citrate utilization, urease, cytochrome oxidase, triple sugar iron (TSI), mannitol fermentation, and production of hydrogen sulphide. Nineteen *Salmonella enterica* were isolated, 12 (63.15%) were *Salmonella typhi* while *Salmonella paratyphi* was only isolated 7(36.83%). The organisms were recovered from patients of all ages between 0 and 70 years, but were primarily isolated from patients aged between 0 and 10 years, as shown in **Table (1).**

For this study, ESßLs *Salmonella enterica* were chosen for molecular identification after being verified through biochemical analysis. These bacteria were identified by their 16S rRNA gene sequence homology, which showed single bands, as seen in **Figure (1).** The 16S rRNA gene sequence was tested by BLAST in the Center for Biotechnology Information (NCBI) database, and a phylogenetic tree was constructed using available reference sequences from the NCBI. It was found that the ESßLs *Salmonella enterica* had a 97% similarity with other *Salmonella enterica* in the NCBI, as displayed in **Figure (2)**.

Prevalence of ESßLs *Salmonella enterica*

The study recruited 669 participants from three tertiary hospitals in Northeast Nigeria. Of these, 373 (55.5%) were females and 296 (44.5%) were males. The study found the overall prevalence of ESßLs *Salmonella enterica* in the study area to be 2.8%. Most of the isolates were obtained from blood samples, as shown in **Table (2.)** Interestingly, the group of participants who were not exposed to antibiotics (2.54%) and those attending the general patient department (1.79%) had the highest prevalence of ESßLs *Salmonella enterica*, as shown in **Table (3)**. In contrast, lower prevalence was found in participants who were exposed to antibiotics (0.29%) and those with gastroenteritis

problems (0.44%). Males showed a higher prevalence (1.49%) compared to females (1.34%). Patients with symptoms of both gastroenteritis and pyrexia had the highest prevalence of ESßLs *Salmonella enterica* (1.68%) compared to patients with only gastroenteritis (0.44%) or pyrexia (0.74%). Among the different age groups, the highest prevalence of ESßLs *Salmonella enterica* species was found in the age group of 0-10 years (1.04%), followed by the age group of 11-20 years (0.74%), and then the age group of 61-70 years (0.59%). The age groups between 31-40 and 41-50 had a prevalence of 0%, as shown in **Table (3)**.

Antimicrobial susceptibility test

The antibiotic susceptibility profile presented in **Table (4**) indicated a high level of antibiotic resistance to first- and second-generation antibiotics including ampicillin, cotrimoxazole and septrin. Members of the Enterobacteriaceae family such as *K. pneumoniae*, *K. oxytoca*, *P. vulgaris*, *P. mirabilis*, *Shigella* sp, Citrobacter *diversus*, *Citrobacter ferundii* and *E. coli* that were isolated in the present study, have exhibited a high level of antibiotic resistance to many of the commonly used antibiotics like penicillin, amoxicillin, ampiclox, septrin, ceporex nalidixic acid and amikacin. Moreover, results indicated that antibiotics with greatest sensitivity against the *Salmonella enterica* isolates were amoxicillin-clavulanic acid 19(100%), ceftazidine 18 (94.7%), meropenem 18 (94.6%), imipenem 17 (89.3%), ceftriaxone 16(69.6%) and ciprofloxacin 14 (73.6%), (table 4.9). However, maximum resistance was observed for ampicilliln 19(100%), amoxicillin 19(100%), amikacin 19(100%), penicillin 19(100%), nalidixic acid 19(100%) respectively. Further, it was found that 12(52.2%) of the isolates showed intermediate sensitivity to chloramphenicol, 65.2% to cefotaxime, 47.8% to doxycycline and 39.1% to ciprofloxacin respectively. Antibiotics amoxicilin-clavulanic acid, ceftazidime, ceftriaxone, meropenem, and imipenem were the most effective against ESßLs *Salmonella enterica* isolated from the three selected tertiary healthcare facilities in Northeast Nigeria. None of the 19 isolates were resistant to amoxicillin-clavulanic acid, imipenem and meropenem.

Detection of ESßLs genes

Detection of ESßLs production potential was performed both phenotypically and genetically. All isolates of *S. enterica* were screened for ESßLs production using a double disc synergy test. Following the NCCLs criteria for the detection of ESßLs production in *E.coli* and *Klebsiella pneumonia*. Nineteen (19) of the *Salmonella enterica* isolates were phenotypically confirmed using DDST as ESßL producers **(Figure 3).** The isolates were further screened for three different extended spectrum βeta-lactamase genes namely; SHV, CTXM-1 and CMY-2 using multiplex PCR. Two (10.50%) of the 19 isolates were genotypically positive for one or more of the resistance genes tested **(Figure 4)**. SHV and CMY-2 were detected in one of the isolates (5.3%). In addition, CTXM-1 was detected in one of the isolates.

Table 1. Percentage of *Salmonella enterica* species isolated according to patients age.

Age	Blood	Stool	Total	No. of ESßLs *S. enterica* isolated	Percentage (%) of ESßLs *S. enterica* Isolated
0-10	147	41	188	7	1.04
11-20	78	29	107	5	0.74
21-30	72	66	138	1	0.14
31-40	25	73	98	0	0
41-50	12	35	47	0	0
51-60	11	20	31	1	0.14
61-70	11	12	23	4	0.59
>71	15	22	37	1	0.14
Total	371	298	669	19	2.84

Table 2. Percentage of *Salmonella enterica* species isolated from blood and stool at the three selected hospitals in the study area.

Hospitals	Number of specimens collected		*Salmonella enterica* species isolated (%)				
	Specimens		*Salmonella typhi*		*Salmonella paratyphi*		Total (%)
	Blood	Stool	Blood	Stool	Blood	Stool	
ATBUTH	116	106	4 (3.4)	3(2.8)	1 (0.86)	2 (1.8)	10 (1.49)
UMTH	150	113	3 (2)	1 (0.88)	1(0.66)	0 (0)	5 (0.89)
FTH	105	79	2 (1.9)	1 (1.2)	0 (0)	1 (1.2)	4 (0.47)
Total	371	298	9(2.4)	5 (1.67)	2 (0.53)	3 (1.06)	
	669		19				2.84

Keys: ATBUTH- Abubakar Tafawa Balewa University Teaching Hospital, UMTH- university of Maiduguri Teaching Hospital, FTH- Federal Teaching Hospital (Gombe**).**

Table 3. Percentage of *S. enterica* species isolated according to patients' clinical information.

Clinical Information	Blood	Stool	Total	No. of *Salmonella enterica* Species Isolated	Percentage *Salmonella enterica* Isolated
Symptoms (%)					
Pyrexia	78 (11.6%)	69 (10.4%)	147 (21.9%)	5	0.74%
Gastroenteritis	126 (18.9%)	92 (13.8)	218 (32.6%)	3	0.44%
Pyrexia and Gastroenteritis	166 (24.9%)	136 (20.3%)	302 (45.2%)	11	1.68%
Gender (%)					
Male	175 (26.1%)	121 (18%)	296 (44.2%)	10	1.49%
Female	196 (29.2%)	177 (26.4%)	373 (56.8%)	9	1.34%
Exposure to antibiotics (%)					
Exposed	312 (46.6%)	263 (39.3%)	575 (85.9%)	2	0.29%
Not exposed	59 (8.8%)	35 (5.2%)	94 (14.05%)	17	2.54%
Group (%)					
Out-patient	214 (30.2%)	186 (27.6%)	400 (59.8%)	12	1.79%
In-patient	157 (2.5%)	112 (16.7%)	269 (40%)	5	0.74%

Table 4. Antibiotic susceptibility test results of *Salmonella* enterica *species* isolated.

Antibiotics	Codes	Conc.	Diameter of zone of inhibition in mm (as per the manufacturer guidelines)		
			≥ **Sensitive%**	**Intermediate%**	≤ **Resistant%**
Amoxi-clav	AMP	25/5µg	19(100%)	0(0%)	0 (0%)
Amoxicillin	AMX	10 µg	0(0%)	0(0%)	19 (100%)
Amikacin	AMK	30 µg	0(0%)	0(0%)	19 (100%)
Ceftazidime	CAZ	30 µg	1 (5.3%)	18 (94.7%)	0(0%)
Ceftriaxone	CTR	30 µg	3 (13.0%)	16(69.6%)	0(0%)
Cefotaxime	CTX	30 µg	6 (31.5%)	13(68.4%)	0(0%)
Ciprofloxacin	CIP	5 µg	0(0%)	14 (73.6%)	5(21.7%)
Chloramphenicol	CHL	30 µg	1(5.4%)	18(94.2%)	0(0%)
Doxycycline	DOX	30 µg	3(15.7%)	11(47.8%)	5(21.7%)
Ampicillin	AMP	10 µg	0 (0%)	0(0%)	19 (100%)
Co-trimoxazole	COT	10 µg	1 (4.3%)	5 (21.7%)	17(74%)
Imipenem	IPM	10 µg	17 (89.3%)	2(10.7%)	0(0%)
Ceporex	CX	5 µg	5(26.3%)	2(10.7%)	12(63.0%)
Meropenem	MEM	10 µg	18 (94.6%)	1(5.4%)	0 (0%)
Nalidixic Acid	NA	30 µg	0(0%)	0(0%)	19(100%)
Pefloxacin	PEF	30 µg	7(36.7%)	12(63.0%)	0(0%)
Oxacillin	OXA	10 µg	1(5.26%)	11(57.8%)	7(36.8%)
Penicillin	PEN	10 µg	0(0%)	0(0%)	19(100%)
Sulphate Colistin	CT	30 µg	1(5.4%)	11(57.8%)	7(36.8%)
Streptomycin	STP	300 µg	2(10.3%)	9(47.4%)	8 (42.3%)
Tetracycline	TET	30 µg	4(21.0%)	1(5.4%)	14(73.6%)
Septrin	SN	30 µg	0(0%)	1(5.4%)	18(94.7%)

Figure 1. Agarose gel electrophoresis for amplified PCR products of 16S rRNA using universal primers (lanes 1-2). Lane 1: M-represents marker. Lane 3: negative control

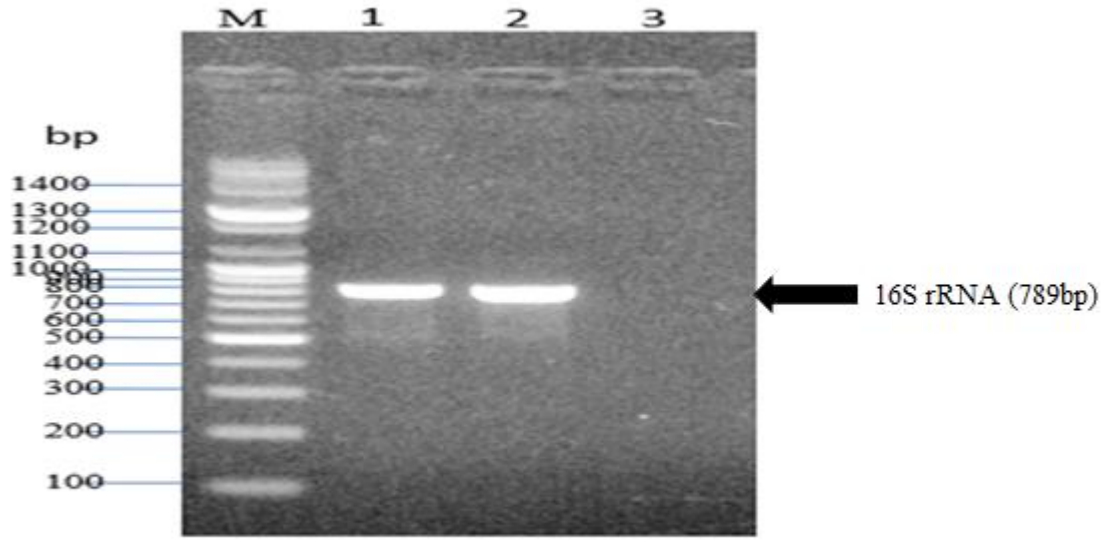


Figure 2. A phylogenetic tree showing relationship between the 16S rRNA gene sequences of isolated ESßLs *S. enterica* strains and that of other *S. enterica* species from NCBI. The ESßLs *S. enterica* isolated were designated as *Salmonella enterica* strain MIA7 and *Salmonella enterica* Strain MIA12. The evolutionary relationship was inferred using Neighbor-joining method. The horizontal bar indicates 0.10.

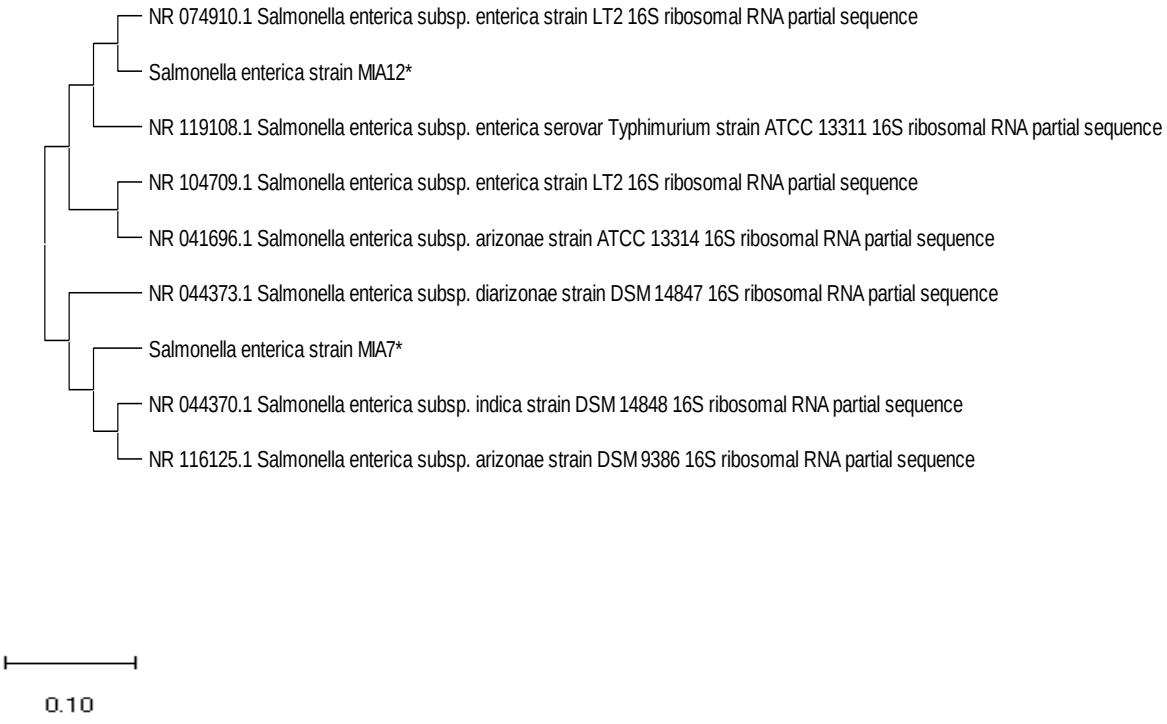
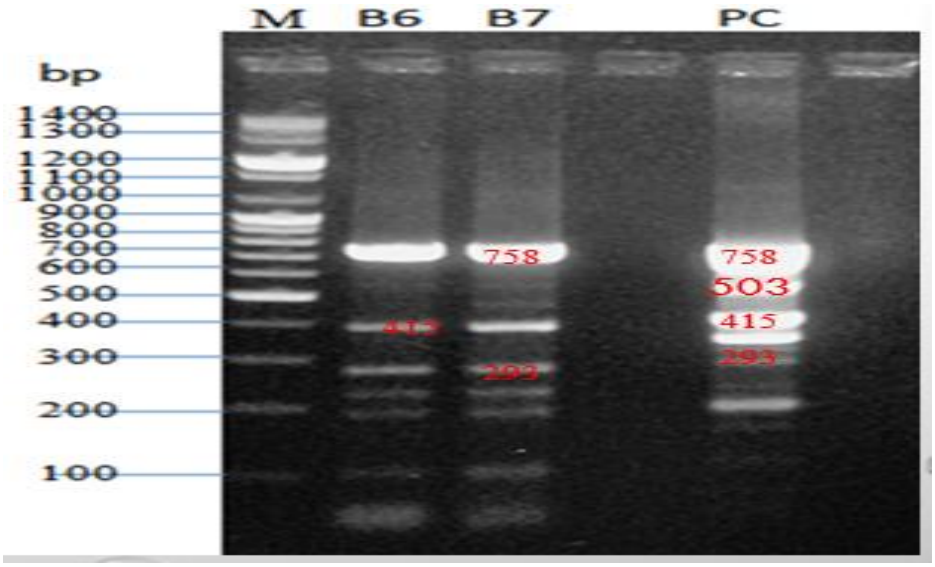


Figure 3. Agarose gel electrophoresis for amplified PCR products using ESßLs genes primers (SHV-293, CTMX-1-415 and CMY-2 758) on ESßLs *Salmonella enterica* species (lanes B6 and B7). Lane 1: m-represents marker, PC: positive control.



Discussion

Our study revealed a prevalence of 2.8% of extended spectrum beta-lactams producing *Salmonella enterica*. The isolates were recovered from blood and stool samples of patients who presented with pyrexia, gastroenteritis or both pyrexia and gastroenteritis from three selected tertiary hospitals in Northeast Nigeria **(Table 1)**. The prevalence of ESßLs *Salmonella enterica*. in this study was relatively low when compared to the results obtained from other studies such as 9.4% recorded in Southeast Nigeria, 16.7% in Northwest Nigeria [10], 24.56% from West Bengal, India, 32.1% recorded in North Karnataka, India, 53% in Mumbai and 62.3% recorded in Pondicherry [24,25, 26]. Evidence from studies has shown that there was an observed relationship between the distribution of *Salmonella* species and the season of the year, with the rainy season having the highest prevalence (May/October) [27,28].

Therefore, the prevalence of *Salmonella enterica* was relatively low in this study due to the fact majority of our samples were collected during the dry season. The area of sample collection being tertiary health care facilities also affected the number of extended spectrum Beta-lactams producing *Salmonella enterica* species isolated at the end of the study. From the clinical information obtained from our study subject, more than 70% of the study participants had been to different health facilities before the tertiary health care where they had been given treatment, and this affected the number of circulating microorganisms in their blood and stool samples even though they have not gotten rid of the infection, which is in line with the findings of [29,10].

The prevalence of ESßLs *Salmonella enterica* based on age in our study is corroborated by the findings of [30] who reported a decrease in ESßLs in older patients which they attributed to immunity in older patients and the work [31]. However, our result contradicts the findings of [32] that shows the age group 65 years and above to have the highest of the total ESßLs *Salmonella enterica* isolated. Our study indicated that as the age increases, the number of ESßLs *Salmonella enterica* species recovered decreases probably due to improved immunity **(Table 4)**. Males recorded a higher prevalence than females, a similar trend was observed by [33] in Benue State where they observed more male patients having a higher prevalence than females. In addition, patients presenting with both pyrexia and gastroenteritis produce more ESßLs *of Salmonella enterica* than others, this conforms with the work of [34] who reported more patients with both gastroenteritis and pyrexia had ESßLs *Salmonella enterica* than others in research conducted in Calabar, Southern Nigeria.

Resistance to antibiotics was mostly observed in *Salmonella enterica* serovar

typhi and *paratyphi*. In the case of our study, *Salmonella enterica* isolates have shown the highest resistance level to ampicillin amoxicillin, amikacin, penicillin, and nalidixic acid respectively. However, some studies conducted around the globe such as one carried out by [9] reported *S. Saintpaul* strains to be susceptible to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfisoxazole, and tetracycline. [35] Reported all isolates of *Salmonella enterica* serovar *javiana* to be sensitive to chloramphenicol, nalidixic acid, and sulfisoxazole, and four isolates showing intermediate resistance to gentamicin or kanamycin. Antimicrobial susceptibility testing of 32 clinical isolates shows that ten (10) of the isolates were sensitive to all eight classes of antibiotics, eleven clinical isolates were resistant to ampicillin, while ten clinical isolates showed resistance to tetracycline. Only one clinical strain was resistant to nalidixic acid [35]. All strains were responsive to the tested carbapenems, which is a significant finding given that carbapenems are the first line of treatment for microorganisms that produce ESßLs.

Due to the selective pressure from the use of extended-spectrum cephalosporin antibiotics, organisms possessing SHV-type were discovered in every continent with a human population during the first ten years of extended-spectrum cephalosporin clinical usage [35]. The enzyme has also been detected in most of the members of the family Enterobacteriaceae, and several studies have reported the outbreak of SHV-producing *Pseudomonas aeruginosa* and *Acinetobacter* spp [9]. In *Salmonella enterica* serovar, ESßLs from the TEM, SHV, and CTX-M β-lactamase families predominate. Of these, CTX-M seems to be the most prevalent. According to studies, individuals who are hospitalized and treated with β-lactam antibiotics like cefoxitin also experience a recovery of Gram-negative bacteria that produce β-lactamases [36].

The prevalence of ESßLs among the *Salmonella enterica* isolated in this study was 19/19 (100%). Studies conducted by [9] reported a prevalence of 40% among *Salmonella typhi* serovars. This has shown an increase in the trend of ESßLs in the clinical settings in our environment due to selective pressure on the use of extended-spectrum cephalosporins in the treatment of enteric fever. Out of the 105 positive cultures, in which 60.9% were Gram-negative bacteria, the Double-Disc Synergy Test (DDST) was used to phenotypically identify 15 (24.5%) of the samples as ESßLs producers [10]. Suggesting that the prevalence of ESßLs in *Salmonella* serotypes has been on the increase when compared to other bacterial pathogens in the family Enterobacteriaceae [37]. Studies conducted by [9,38] reported 9.3% and 15.8%, respectively, ESßLs prevalence in Gram-negative bacteria.

The present investigation revealed a significant difference in the detection of ESßLs genes among the isolates as only 2 (10.5%) of the isolated organisms were found to harbor three types of the ESßLs genes namely- CMY-2, CTXM-1 and SHV after confirming through molecular method. However, the initial phenotypic investigation has shown that all the nineteen isolates were found to demonstrate ESßLs activity. Phenotypic methods for ESßLs detection can only detect the presence of an ESßL; they cannot identify its subtype or identify genes whose expression is suppressed or masked [39]. Only molecular detection techniques can provide a definitive identification of ESßLs genes [40].

Conclusion

This study has highlighted the burden of ESßLs among *Salmonella enterica* species in our environment with a general prevalence of 2.8%. About 10% of the phenotypically confirmed isolates were found to contain three ESßL genes, namely CMY-2, CTXM-1, and SHV, using multiplex PCR. Results from the antimicrobial resistance pattern showed that all the isolates of *Salmonella enterica* were sensitive to meropenem, imipenem and amoxicillin-clavulanic acid, but were resistant to more than three (3) classes of antibiotics. All strains were responsive to the tested carbapenems, which is a significant finding given that carbapenems are the first line of treatment for microorganisms that produce ESßLs. The presence of ESßLs has contributed to the multidrug resistance of the isolates.

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Author's contribution

Adamu, M.B. designed the study design and searched for literatures

Moi I.M. reviewed and edited the manuscript.

Adedeji I. contributed in the collection and processing of the clinical samples.

Uba A. supervised the research work.

Adamu S.U. revised the manuscript

All authors contributed to the final version of the manuscript

Conflict of interest statement

The authors declare that there is no conflict of interest and that the manuscript has not been published so far or communicated to any other journal. The manuscript has been read and approved by all the authors and that the requirement for authorship as stated earlier in the document have been met and that each author believes the manuscript is an honest work.

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